**A Study on Qualitative and Quantitative Phytochemical Analysis of Selected True and Associate Mangroves**

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ABSTRACT

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| **Aims:** To determine the qualitative and quantitative analysis of various plant parts by using the solvents of varying polarity.  **Place and Duration of Study:** Plant Physiology Laboratory, Department of Botany and Microbiology, Acharya Nagarjuna University, during May and June, 2022.  **Methodology:** True mangroves i.e. *Aegiceras corniculatum* (*Ac*) and *Sonneratia apetala* (*Sa*)) and associate mangrove species i.e. *Clerodendrum inerme* (*Ci*), *Derris trifoliata* (*Dt*) and *Suaeda maritima* (*Sm*) of Nizampatnam located on the south-east coast of Andhra Pradesh, India were selected for the study. Healthy and fresh leaves, stems, and roots were washed and dried. All the plant parts powdered with the help of a food processor and sieved. The powders were tested for qualitative and quantitative screening of phytochemicals hexane, ethyl acetate, methanol, and water.  **Results:** Qualitative and quantitative investigations found that the true mangrove, *Aegiceras corniculatum*, showed a diverse range of phytochemicals. The methanol and ethyl acetate extracts were especially high in phenolics (53.01 mg/g), coumarins (24.81 mg/g), flavonoids (63.47 mg/g), saponins (32.07 mg/g), tannins (51.17 mg/g), and terpenoids (56.11 mg/g). Mangrove associate *Suaeda maritima* outperformed the other mangrove associates. *S. maritima* contained high amounts of saponins (18.23 mg/g), terpenoids (52.66 mg/g), flavonoids (16.32 mg/g), and alkaloids (6.87 mg/g).  **Conclusion:** True mangrove *Aegiceras corniculatum* displaying the highest phytochemical potential both qualitatively and quantitatively. Mangrove associate *Suaeda maritima* also shown outstanding phytochemical richness and yield among the related associate mangroves. |

*Keywords: Phytochemicals, Mangroves, Mangrove associates, Solvents, Nizampatnam*

1. INTRODUCTION

Non-communicable diseases (NCDs) such as cardiovascular disease, cancer, diabetes, and chronic respiratory disorders will continue to be important global health issues in 2024. NCDs are projected to account for 74% of all deaths globally, with 41 million people dying each year, 15 million of whom are between the ages of 30 and 69 (https://combatinglungcancerasiapacific.com). It has been estimated that 77% of this burden was deflected on to low- and middle-income nations (GBD 2019 Collaborators, 2020). The burden is most severe in India, where NCDs have caused almost 63% of all deaths. Cardiovascular diseases alone account for approximately 28% of all Indian deaths, representing more than 3 million deaths each year, while the diabetic population has increased to 212 million, representing almost 26% of the world diabetic population (Sweta *et al*., 2024).

The increasing cases of drug-resistant diseases and long-term side effects emphasize the imminent need to investigate alternative, safer, and sustainable therapeutic strategies. Allopathic traditional medicine, while successful in acute treatment and symptomatic cure, frequently suffers severe constraints in long-term health outcomes. Extended use of synthetic medicines correlates with organ toxicity, antimicrobial resistance, drug adverse effects, and iatrogenic complications (Lazarou *et al*., 1998; Ventola, 2015) as well as expensive treatment. On the contrary, herbal plant medicines have re-emerged as a vital component of therapy based on their biocompatibility, low cost, and wide pharmacological activity. The World Health Organization (WHO) approximates that more than 80% of the global population depends on traditional plant medicines for primary healthcare, especially in Africa and Asia (WHO, 2021). Phytochemicals present extensively in medicinal plants, display major antioxidant, anti-inflammatory, anticancer, antimicrobial, and immunomodulatory activities (Atanasov *et al*., 2021). Mangrove ecosystems, which cover intertidal areas along tropical and subtropical shores, are perhaps the most productive and ecologically important biomes on Earth. These specialized vegetation communities have developed distinctive physiological and structural features to live in saline, hypoxic, and highly dynamic habitats (Kathiresan and Bingham, 2001; Tomlinson, 2016). Mangrove vegetation is generally divided into true mangroves, which are obligate and restricted to mangrove swamps, and associate mangroves, which are facultative species with distributions in both mangrove and non-mangrove sites (Duke, 1992). Aside from their biological roles in shoreline protection, carbon fixation, and nutrient cycling, mangroves are now valued for their abundance of bioactive secondary metabolites. These phytochemicals, which include flavonoids, phenolics, alkaloids, tannins, and terpenoids, play essential roles in plant defense, allelopathy, and stress tolerance. Furthermore, various mangrove elements have demonstrated interesting pharmacological properties, including antioxidant, antibacterial, anti-inflammatory, and anticancer action (Bandaranayake, 2002; Kathiresan and Rajendran, 2005).

Mangrove habitats are dwell in typical environmental conditions, such as salt, UV irradiaion, and oxidative stress. To cope of these adverse situations mangroves promote the manufacture of secondary metabolites, contributing to their specific phytochemical compositions (Alongi, 2002). Despite this potential, comparative phytochemical studies between true and associate mangrove species remain limited, especially with regard to both qualitative and quantitative profiling. This study aims to analyze the phytochemical content of selected true and associate mangrove species using standard qualitative assays and spectrophotometric quantification. Further this study highlight their relevance in drug discovery, natural product research, and conservation biology.

2. material and methods

True mangroves i.e. *Aegiceras corniculatum* (*Ac*) and *Sonneratia apetala* (*Sa*)) and associate mangrove species i.e. *Clerodendrum inerme* (*Ci*), *Derris trifoliata* (*Dt*) and *Suaeda maritima* (*Sm*) of Nizampatnam located on the south-east coast of Andhra Pradesh, India were selected for the study. Healthy and fresh leaves, stems, and roots were washed and dried. All the plant parts powdered with the help of a food processor and sieved. The powders were tested for qualitative and quantitative screening of phytochemicals hexane, ethyl acetate, methanol, and water.

**2.1 Plant Collection and Sample Preparation**

Plant samples were collected from the middle and upper canopy during May and June 2022. The collected samples were washed thoroughly to remove surface impurities. Subsequently, the rinsed samples subjected to shade dry, followed by pulverization using a mechanical blender. The resulting powder was filtered through a 0.25 mm sieve to ensure uniform particle size. The obtained powders were stored in airtight containers until further use.

**2.2 Extraction of Plant Material**

Approximately 150 g of powdered leaf, stem, and root material from each plant species was subjected to Soxhlet extraction solvents of increasing polarity i.e n-hexane, ethyl acetate, 80% methanol, and double-distilled water. Extraction was continued up to 12–18 hours. The resulting crude extracts were concentrated using a rotary vacuum evaporator (Buchi Labortechnik AG, Model R-215). The dried extracts were stored at 4 °C in sealed glass vials for subsequent phytochemical screening.

**2.3 Preliminary Phytochemical Screening**

Qualitative phytochemical screening of crude extracts was performed using standard protocols of Harborne, (1973) and Gibbs, (1974) to determine the presence phenolic compounds, coumarins, cardiac glycosides, saponins, tannins, flavonoids, terpenoids and alkaloids.

**2.3.1 Phenolic Compounds**: To 2 mL of the test extract, 0.5 mL of ferric chloride solution (FeCl3) (w/v) was added. The appearance of a dark blue colour showed the presence of phenolic compounds.

**2.3.2 Coumarins**: To a small amount of plant extract alcoholic sodium hydroxide was added and development of a yellow coloration reported the occurrence of coumarins.

**2.3.3 Cardiac Glycosides**: Anthrone reagent was added to plant extract, followed by addition of concentrated sulfuric acid (H₂SO₄) and gently warmed. The formation of deep green coloration confirmed the presence of cardiac glycosides.

**2.3.4 Saponins**: Plant extract was mixed with 10 mL of distilled water and shaken vigorously for 10 minutes. The formation honeycomb-like froth indicates saponins.

**2.3.5 Tannins**: To 2 mL of plant extract, a few drops of lead acetate solution (10%) was added. The appearance white precipitate confirmed the presence of tannins.

**2.3.6 Flavonoids**: The plant extract was mixed with a few magnesium flakes and a few drops of concentrated hydrochloric acid (HCl). The formation of pink coloration indicated the presence of flavonoids.

**2.3.7 Terpenoids**: Plant extract was added with acetic anhydride and concentrated HCl, followed by the addition of 50% sulfuric acid (H2SO4). Formation of blue-green indicated the presence of terpenoids.

**2.3.8 Alkaloids**: To 2 mL of plant extract, a few drops of dilute hydrochloric acid (HCl) was added. Subsequently, 1 mL of Dragendorff’s reagent was added. The appearance of reddish-brown precipitate showed the presence of alkaloids.

Bottom of Form

**2.4. Quantitative phytochemical analysis of leaf extracts**

A quantitative examination of phytochemical components was performed.

**2.4.1 Total phenolic compounds (mg of GA/g):** The phenolic content was measured according to Alhakmani *et al*. (2013). A reaction mixture was prepared by adding 0.5 mL of plant extract, 2.5 mL of 10% aqueous Folin-Ciocalteu's reagent, and 2.5 mL of 7.5% aqueous NaHCO3. The samples were incubated at 45 °C for 45 minutes. The absorbance was reocrded at 765 nm. A standard was prepared using gallic acid.

**2.4.2 Coumarins (mg CE/g):** Coumarin content was measured using the Rajat Buragohain (2015) method. To the plant extract, 2 ml of distilled water and 500 µl of a 5% lead acetate solution (w/v) was added. Contents were shook vigorously, followed by the addition of 7 ml of distilled water and mixed well. From this solution 2 ml was taken into separate test tube, and added with 8 ml of 0.1 M (v/v) hydrochloric acid. The solution was maintained at room temperature for 30 minutes and the absorbance was measured at 320 nm.

**2.4.3 Cardiac glycosides (g/100 g):** Cardiac glycosides were determined according to Muhammad and Abubakar (2016). To the 8 ml of plant extract 60 ml of H2O and 8 ml of 12.5% lead acetate was added, and then filtered. From this, 50 ml of the filtrate was transferred to another 100 ml flask. To this 8 ml of 47% Na2HPO4 was added in order to precipitate the excess Pb2+ ions and diluted with water. The mixture was filtered twice. Now, 10 mL of filtrate was transferred to a clean Erlyn-Meyer flask and treated with 10 mL of Baljet reagent. A blank titration was performed using 10 mL of pure water and 10 mL of Baljet reagent. This was allowed to stand for one hour and the color intensity was determined at 495 nm.

**Optical Density × 100**

**% Total glycosides = ----------------------------------**

**77**

**2.4.4 Total saponins (%):** The saponin content was calculated according to Aryan *et al*. (2022). The plant extract was mixed with 100 ml of aqueous ethanol (20%). The solution was heated at 55 °C for 4 hours, with constant stirring. The solution was filtered and extracted using 200 mL of ethanol (20%). Both extracts were mixed, and the solvent was evaporated to reach 40 mL. This was further extracted to 20 mL using diethyl ether. The aqueous layer was retained, and purified with 60 mL of n-butanol. It was then rinsed with 10 mL of aqueous NaCl (5%). The purified plant sample was measured at 380 nm.

**2.4.5 Tannin (mg TA/g):** The tannin content was measured using Folin-Ciocalteu method (Polshettiwar *et al*., 2007). To 0.1 ml of plant extract, 7.5 ml of distilled water, Folin-Ciocalteu phenol (0.5 ml), and 1 ml of 35% sodium carbonate was added. These contents were diluted to 10 ml. The reaction mixture was thoroughly agitated, incubated for halften hour. The absorbance was determined at 700 nm.

**2.4.6 Flavanoids (mg QE/g):** The total flavonoid content was calculated using quercetin as the standard calibration curve (Igbinosa *et al*., 2013). A 0.5 ml amount of 2% AlCl3 ethanol solution was mixed with 0.5 ml of plant extract. After 1 hour of incubation absorbance was recorded at 420 nm.

**2.4.7 Terpenoids (µg/mg):** The terpenoids were estimated according to Truong *et al*. (2021). A reaction cocktail of 4 N sulphuric acid (2 ml), 0.5% w/v iron (III) chloride (2 ml), and 0.5% w/v potassium hexacyanoferrate (0.5 ml) was added to 1 ml of plant extract. The mixture was heated for 30 minutes at 70 ⁰C and absorbance was measured at 780 nm.

**2.4.8 Alkaloids (mg of AE/g):** One milligram of plant extract was dissolved in dimethyl sulfoxide (DMSO), followed by the addition of one milliliter of 2N HCl and filtered. This solution was further added to a separate funnel containing 5 ml of bromocresol green solution and 5 ml of phosphate buffer. The mixture was agitated with 1, 2, 3, and 4 mL of chloroform, and collected in a 10 mL volumetric flask containing chloroform. The reference standard solutions of atropine (20, 40, 60, 80, and 100 µg/ml) were prepared. The optical density of plant extract and standard solutions was measured at 470 nm.

3. results

The qualitative screening of phytochemicals in the leaves, stems, and roots of selected true and associate mangrove species *Aegiceras corniculatum* (*Ac*), *Clerodendrum inerme* (*Ci*), *Derris trifoliata* (*Dt*), *Suaeda maritima* (*Sm*), and *Sonneratia apetala* (*Sa*) was conducted using four solvents of varying polarity i.e. hexane, ethyl acetate, methanol, and water (Table 1). The presence of phenolic compounds was recorded in the leaf extracts of all species, with the exception of the hexane and ethyl acetate extracts of *C. inerme*. All stem extracts contained phenolics, except for the ethyl acetate extract of *D. trifoliata* and aqueous extracts of *A. corniculatum* and *S. maritima*. In roots, phenolics were detected in all plant samples when extracted with methanol. However *C. inerme* of hexane, ethyl acetate and methanol also not reported phenolic compounds. Their presence also not observed in *D. trifoliata* samples of ethyl acetate. Coumarins were widely distributed across leaf extracts, except in the hexane, ethyl acetate, and aqueous extracts of *C. inerme*, and the methanol extract of *D. trifoliata*. In stem extracts, ethyl acetate consistently revealed the presence of coumarins in all species, whereas hexane extract of *C. inerme*, and methanol extracts of *A. corniculatum*, *S. apetala*, and *S. maritima* also tested positive. Notably, aqueous stem extracts of all species failed to show coumarins. Similarly, coumarins were absent in root extracts of *C. inerme* (all solvents), *D. trifoliata* (hexane, ethyl acetate, and aqueous), and *A. corniculatum* (hexane and aqueous). Glycosides were present in leaf extracts of all species and solvents, except in *D. trifoliata* extracts with hexane, methanol, and water. In stem tissues, glycosides were absent in the ethyl acetate and aqueous extracts of *C. inerme*, and in the aqueous extract of *S. maritima*. In root samples, glycosides were only detected in the hexane extract of *A. corniculatum*, while the ethyl acetate, methanol, and aqueous extracts of *C. inerme*, *D. trifoliata*, and *S. maritima* tested negative. Saponins were universally detected in leaf extracts across all solvent types. In stem samples, saponins were present in all species except *A. corniculatum*. Root extracts of *C. inerme* (hexane, ethyl acetate, and aqueous) and *D. trifoliata* (aqueous) did not exhibit saponin presence. Tannins were detected in all leaf extracts prepared with methanol and water, while the hexane extract was positive only for *A. corniculatum*. Ethyl acetate extracts showed tannin presence in all species except *C. inerme*. All stem extracts revealed tannin content except for the aqueous extract of *A. corniculatum*. In roots, tannins were not observed in *C. inerme* (ethyl acetate, methanol, and aqueous) and in the hexane extract of *D. trifoliata*. Flavonoids were present in nearly all leaf extracts, with exceptions noted in the hexane extracts of *D. trifoliata*, *S. apetala*, and *S. maritima*, and in the ethyl acetate and aqueous extracts of *S. maritima*. In stem extracts, flavonoids were observed in all samples except the aqueous extracts of *A. corniculatum* and *S. maritima*. All root extracts across solvents confirmed the presence of flavonoids. Terpenoids were broadly distributed across all root samples irrespective of the solvent. In leaf extracts, terpenoids were absent in the hexane and aqueous extracts of *A. corniculatum*, and in the ethyl acetate and aqueous extracts of *S. maritima*. In stem extracts, terpenoids were not detected in the hexane and aqueous extracts of *S. maritima*, and in the aqueous extract of *A. corniculatum*. Alkaloids were not detected in the aqueous leaf extracts of *A. corniculatum* and *S. maritima*. Similarly, aqueous stem extracts of *A. corniculatum*, *D. trifoliata*, and *S. maritima*, and the hexane extract of *S. maritima*, did not exhibit alkaloid presence. In roots, alkaloids were absent in the aqueous extracts of *C. inerme* and *D. trifoliata*.

**Table 1. Phyotchemical screening of mangroves and associate mangroves**

| **Phytochemicals** | **Solvent** | ***Aegiceras corniculatum*** | ***Clerodendrum inerme*** | ***Derris trifoliata*** | ***Suaeda maritima*** | ***Sonneratia apetala*** |
| --- | --- | --- | --- | --- | --- | --- |
| **Phenolic Compounds** | Hexane | + | – | + | + | + |
|  | Ethyl acetate | + | – | + | + | + |
|  | Methanol | + | + | + | + | + |
|  | Water | + | + | + | + | + |
| **Coumarins** | Hexane | + | – | + | + | + |
|  | Ethyl acetate | + | – | + | + | + |
|  | Methanol | + | + | – | + | + |
|  | Water | + | – | + | + | + |
| **Glycosides** | Hexane | + | + | – | + | + |
|  | Ethyl acetate | + | + | + | + | + |
|  | Methanol | + | + | – | + | + |
|  | Water | + | + | – | + | + |
| **Saponins** | Hexane | + | + | + | + | + |
|  | Ethyl acetate | + | + | + | + | + |
|  | Methanol | + | + | + | + | + |
|  | Water | + | + | + | + | + |
| **Tannins** | Hexane | + | – | – | – | – |
|  | Ethyl acetate | + | – | + | + | – |
|  | Methanol | + | + | + | + | + |
|  | Water | + | + | + | + | + |
| **Flavonoids** | Hexane | + | + | – | – | – |
|  | Ethyl acetate | + | + | + | + | + |
|  | Methanol | + | + | + | + | + |
|  | Water | + | + | + | + | + |
| **Terpenoids** | Hexane | – | + | + | + | + |
|  | Ethyl acetate | + | + | + | + | – |
|  | Methanol | + | + | + | + | + |
|  | Water | – | + | + | + | – |
| **Alkaloids** | Hexane | + | + | + | + | + |
|  | Ethyl acetate | + | + | + | + | + |
|  | Methanol | + | + | + | + | + |
|  | Water | + | + | – | + | – |

The potential yield of phytochemicals of the leaves, stems, and roots of test plants *Aegiceras corniculatum (Ac), Clerodendrum inerme (Ci), Derris trifoliata (Dt), Suaeda maritima (Sm),* and *Sonneratia apetala (Sa)* was assessed by using four solvents with varied polarity i.e hexane, ethyl acetate, methanol, and water (Table 2). The data revealed considerable variation in the extraction efficiency of different phytochemicals across species, plant parts, and solvents. In present study phenolic compounds were effectively extracted with methanol. Among the leaf samples, *S. apetala* reported the high phenolic content in methanol extract (41.21 mg/g), whereas the minimum content was observed in hexane extract of *D. trifoliata* (3.67 mg/g). In stem tissues, phenolic content ranged from 0.89 mg/g in hexane extract of *C. inerme* to 52.11 mg/g in methanol extract of *A. corniculatum*. Root extracts also followed a similar trend, with *A. corniculatum* methanol extract yielding the highest phenolic content (53.01 mg/g), while the methanolic extract of *C. inerme* yielded the lowest (1.72 mg/g). Coumarin content was highest in *A. corniculatum* across all parts, with leaf extracts showing 22.16 mg/g in ethyl acetate and 19.25 mg/g in methanol rxtract. The lowest coumarin level in leaf tissues was noted in methanol extract of *C. inerme* (2.67 mg/g). In stem samples, maximum coumarin concentrations were again observed in methanol (18.27 mg/g) and ethyl acetate (18.21 mg/g) extracts of *A. corniculatum*, while *C. inerme* hexane extract exhibited the lowest content (2.81 mg/g). Among root samples, *A. corniculatum* showed highest yields 24.81 and 24.14 mg/g in methanol and ethyl acetate, respectively, with the lowest in methanol extract of *C. inerme* (1.98 mg/g). Glycoside extraction showed solvent-dependent variability. Ethyl acetate effectively extracted glycosides from all leaf samples, with the highest content in *A. corniculatum* methanol extract (6.89 mg/g) and the lowest in aqueous extract of *C. inerme* (0.98 mg/g). Similar trends were observed in stems and roots, with *A. corniculatum* methanol extracts yielding higher glycoside levels 5.11 mg/g in stem and 5.84 mg/g in root respectively, whereas *C. inerme* and *A. corniculatum* hexane extracts yielded the lowest 0.76 and 1.98 mg/g, respectively both in stem and roots. Saponins were consistently extracted by all solvents from leaf samples, with *A. corniculatum* showing the maximum levels in all solvents, notably 21.41 mg/g in methanol. The lowest leaf saponin content was found in *C. inerme* hexane extract (2.63 mg/g). In stem samples, saponin content recorded high at 26.20 mg/g in *A. corniculatum* methanol extract and was lowest in *C. inerme* hexane extract (1.00 mg/g). Similar trend was observed in root samples, with *A. corniculatum* methanol extract containing the peak saponin level (32.07 mg/g), and *C. inerme* methanol extract the minimum levels (1.42 mg/g). Tannin content was highest in ethyl acetate extract of *D. trifoliata* leaves (40.61 mg/g), followed by methanol extract of *A. corniculatum* (34.27 mg/g). The lowest yield was reported with aqueous extract of *C. inerme* (4.60 mg/g). In stem samples, *A. corniculatum* methanol and ethyl acetate extracts recorded 46.11 and 40.20 mg/g, respectively. *C. inerme* hexane extract showed the minimum yield (1.47 mg/g). All root samples yielded tannins, with *A. corniculatum* methanol and ethyl acetate extracts showing the highest levels (51.17 mg/g), and *C. inerme* aqueous extract detected with less tannins (1.27 mg/g). Flavonoid content was highest in ethyl acetate extracts of leaves, with *A. corniculatum* exhibiting the highest (63.47 mg/g), and *C. inerme* hexane extract the less content (6.22 mg/g). Notably, hexane extracts of *D. trifoliata*, *S. apetala*, and *S. maritima* did not yield detectable flavonoids. In stems, *A. corniculatum* (22.18 mg/g) and *S. apetala* (21.38 mg/g) methanol extracts showed high flavonoid content, while *C. inerme* hexane extract recorded the lowest flavonoids (4.10 mg/g) levels. Root samples showed highest flavonoid yields in *A. corniculatum* methanol (36.16 mg/g) and hexane (31.64 mg/g) extracts. The lowest content was observed in *C. inerme* ethyl acetate extract (3.10 mg/g). Terpenoid content in leaf samples was highest in methanol (48.12 mg/g) and ethyl acetate (45.21 mg/g) extracts of *A. corniculatum*, while *C. inerme* aqueous extract was recorded the minimum levels of terpenoids (3.22 mg/g). Among stem samples, methanol extracts of *A. corniculatum* (56.11 mg/g) and *S. maritima* (52.66 mg/g) showed higher yields, whereas ethyl acetate extract of *S. maritima* was recorded lower levels (3.10 mg/g). All root extracts yielded terpenoids, with considerable levels across all samples. Alkaloid yield was observed in all leaf extracts, with maximum content in *S. maritima* methanol extract (6.87 mg/g), followed by *A. corniculatum* and *S. apetala* (5.62 mg/g each). *C. inerme* hexane extract yielded the least levels of alkaloids (0.94 mg/g). In stem tissues, methanol (9.12 mg/g) and ethyl acetate (7.27 mg/g) extracts of *A. corniculatum* recorded the high alkaloid contents. No alkaloids were detected in hexane extract of *S. maritima* and aqueous extracts of *A. corniculatum* and *S. maritima*. Among roots, all solvents extracted alkaloids from all the samples, except aqueous extracts of *C. inerme* and *D. trifoliata*. The highest root alkaloid levels were found in *S. apetala* (12.80 mg/g) and *A. corniculatum* (10.12 mg/g) methanol extracts, whereas the minimum levels were recorded in *C. inerme* hexane extract (1.00 mg/g).

**Table 2. Quantification of phytochemicals from mangroves and associate mangroves**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Phytochemical** | **Plant Part** | **Solvent** | ***Ac*** | ***Ci*** | ***Dt*** | ***Sa*** | ***Sm*** |
| **Phenolic compounds** | Leaf | Hexane | 16.60 | - | 3.67 | 4.57 | 4.10 |
| Ethyl acetate | 28.10 | - | 20.19 | 26.11 | 27.80 |
| Methanol | 38.18 | 14.00 | 7.18 | 41.21 | 22.10 |
| Water | 21.11 | 18.10 | 5.16 | 20.16 | 19.35 |
| **Coumarins** | Leaf | Hexane | 12.32 | - | 10.12 | 10.58 | 9.22 |
| Ethyl acetate | 22.16 | - | 16.19 | 16.11 | 14.26 |
| Methanol | 19.25 | 2.67 | - | 18.10 | 16.27 |
| Water | 8.25 | - | 6.58 | 6.89 | 6.10 |
| **Glycosides** | Leaf | Hexane | 3.80 | 0.99 | - | 3.20 | 3.01 |
| Ethyl acetate | 5.20 | 0.98 | 5.11 | 4.01 | 3.94 |
| Methanol | 6.89 | 1.11 | - | 5.94 | 4.17 |
| Water | 4.21 | 0.98 | - | 3.99 | 3.76 |
| **Saponins** | Leaf | Hexane | 12.69 | 2.63 | 5.00 | 6.94 | 5.20 |
| Ethyl acetate | 18.32 | 3.94 | 6.47 | 11.09 | 8.45 |
| Methanol | 21.41 | 5.62 | 9.49 | 13.80 | 12.60 |
| Water | 17.58 | 2.86 | 5.94 | 9.11 | 7.60 |
| **Tannins** | Leaf | Hexane | 30.44 | - | - | - | - |
| Ethyl acetate | 31.54 | - | 40.61 | 26.34 | - |
| Methanol | 34.27 | 14.40 | 32.01 | 33.62 | 31.46 |
| Water | 29.48 | 4.60 | 19.40 | 24.52 | 22.17 |
| **Flavonoids** | Leaf | Hexane | 17.10 | 6.22 | - | - | - |
| Ethyl acetate | 63.47 | 27.19 | 22.11 | 47.99 | 42.86 |
| Methanol | 41.15 | 9.16 | 40.70 | 41.07 | 38.94 |
| Water | 19.12 | 8.75 | 9.26 | 18.11 | 17.68 |
| **Terpenoids** | Leaf | Hexane | - | 4.24 | 18.12 | 20.58 | 17.66 |
| Ethyl acetate | 45.21 | 5.34 | 24.32 | 34.20 | - |
| Methanol | 48.12 | 8.56 | 27.95 | 41.62 | 39.58 |
| Water | - | 3.22 | 12.10 | 14.69 | - |
| **Alkaloids** | Leaf | Hexane | 3.96 | 0.94 | 2.67 | 3.72 | 3.11 |
| Ethyl acetate | 4.89 | 1.04 | 2.69 | 4.44 | 4.10 |
| Methanol | 5.62 | 1.54 | 2.92 | 5.62 | 6.87 |
| Water | 4.11 | 1.00 | 1.41 | 4.59 | 3.11 |
| **Phenolic compounds** | Stem | Hexane | 8.10 | 0.89 | 4.16 | 6.27 | 5.00 |
| Ethyl acetate | 9.67 | 1.03 | - | 38.21 | 39.17 |
| Methanol | 52.11 | 1.89 | 28.10 | 41.90 | 40.27 |
| Water | - | 1.11 | 2.18 | 20.12 | - |
| **Coumarins** | Stem | Hexane | - | 2.81 | - | - | - |
| Ethyl acetate | 18.21 | - | - | 14.10 | 12.17 |
| Methanol | 18.27 | - | - | 15.36 | 12.01 |
| Water | - | - | - | - | - |
| **Glycosides** | Stem | Hexane | 2.80 | 0.76 | 2.10 | 1.89 | 3.00 |
| Ethyl acetate | 4.17 | - | 3.11 | 3.14 | 3.15 |
| Methanol | 5.11 | 0.91 | 3.25 | 4.27 | 3.29 |
| Water | 4.10 | - | 2.98 | 3.51 | - |
| **Saponins** | Stem | Hexane | 9.42 | 1.00 | 3.99 | 9.60 | 7.28 |
| Ethyl acetate | 16.22 | 2.14 | 5.80 | 15.27 | 13.06 |
| Methanol | 26.20 | 2.98 | 6.98 | 7.36 | 7.01 |
| Water | - | 1.89 | 4.11 | 9.74 | 8.66 |
| **Tannins** | Stem | Hexane | 26.20 | 1.47 | 20.14 | 17.26 | 17.10 |
| Ethyl acetate | 40.20 | 3.11 | 20.60 | 37.89 | 36.62 |
| Methanol | 46.11 | 4.89 | 26.10 | 32.11 | 39.60 |
| Water | - | 2.99 | 14.21 | 20.18 | 17.11 |
| **Flavonoids** | Stem | Hexane | 18.42 | 4.10 | 14.30 | 16.38 | 15.10 |
| Ethyl acetate | 19.27 | 4.80 | 14.12 | 16.11 | 16.17 |
| Methanol | 22.18 | 5.50 | 14.40 | 21.38 | 20.66 |
| Water | - | 4.98 | 12.07 | 16.74 | - |
| **Terpenoids** | Stem | Hexane | 28.14 | 14.20 | 20.11 | 20.94 | - |
|  | Ethyl acetate | 50.27 | 6.21 | 26.17 | 39.48 | 3.10 |
|  | Methanol | 56.11 | 10.27 | 35.07 | 52.66 | 48.11 |
|  | Water | - | 3.69 | 14.27 | 20.40 | - |
| **Alkaloids** | Stem | Hexane | 6.10 | 0.74 | 3.00 | 4.67 | - |
| Ethyl acetate | 7.27 | 0.86 | 1.89 | 5.66 | 5.72 |
| Methanol | 9.12 | 0.94 | 2.57 | 7.61 | 6.44 |
| Water | - | 0.79 | - | 4.94 | - |
| **Phenolic compounds** | Root | Hexane | 15.62 | - | 5.40 | 7.89 | 6.12 |
| Ethyl acetate | 40.52 | - | - | 40.27 | 42.40 |
| Methanol | 53.01 | 1.72 | 10.84 | 46.61 | 42.21 |
| Water | 18.12 | - | 4.25 | 24.16 | 20.56 |
| **Coumarins** | Root | Hexane | 14.68 | - | - | 12.01 | 10.65 |
| Ethyl acetate | 24.81 | - | 17.54 | 17.99 | 16.00 |
| Methanol | 24.14 | 1.98 | 14.25 | 19.54 | 17.66 |
| Water | - | - | 6.22 | 7.95 | 7.15 |
| **Glycosides** | Root | Hexane | 1.98 | - | - | - | - |
| Ethyl acetate | 3.65 | - | - | 2.99 | 2.85 |
| Methanol | 5.84 | - | - | 2.44 | 2.41 |
| Water | 2.16 | - | - | 2.45 | 2.00 |
| **Saponins** | Root | Hexane | 18.27 | - | 3.00 | 10.11 | 9.20 |
| Ethyl acetate | 19.01 | - | 4.72 | 17.99 | 16.27 |
| Methanol | 32.07 | 1.42 | 5.47 | 9.09 | 9.01 |
| Water | 14.80 | - | - | 10.40 | 9.27 |
| **Tannins** | Root | Hexane | 18.19 | - | - | 20.10 | 21.44 |
| Ethyl acetate | 41.99 | - | 21.42 | 39.00 | 37.01 |
| Methanol | 51.17 | - | 14.61 | 36.27 | 35.64 |
| Water | 31.47 | 1.27 | 10.42 | 21.27 | 18.28 |
| **Flavonoids** | Root | Hexane | 31.64 | 3.28 | 10.19 | 20.54 | 19.10 |
| Ethyl acetate | 21.66 | 3.10 | 10.23 | 18.20 | 17.34 |
| Methanol | 36.16 | 4.00 | 9.77 | 29.45 | 27.62 |
| Water | 27.01 | 3.15 | 8.99 | 20.00 | 19.47 |
| **Terpenoids** | Root | Hexane | 30.27 | 3.01 | 14.20 | 22.62 | 20.99 |
| Ethyl acetate | 52.02 | 3.26 | 14.66 | 42.17 | 36.23 |
| Methanol | 59.02 | 4.79 | 14.61 | 53.27 | 51.36 |
| Water | 34.56 | 3.11 | 10.27 | 22.15 | 19.40 |
| **Alkaloids** | Root | Hexane | 7.89 | 1.00 | 2.62 | 6.21 | 6.29 |
| Ethyl acetate | 8.14 | 1.20 | 2.19 | 7.86 | 6.94 |
| Methanol | 10.12 | 1.01 | 2.09 | 12.80 | 11.74 |
| Water | 6.11 | - | - | 5.00 | 4.92 |

**4. DISCUSSION**

The present study reveals significant qualitative and quantitative differences in phytochemical composition across five selected true and associate mangrove species *Aegiceras corniculatum*, *Clerodendrum inerme*, *Derris trifoliata*, *Suaeda maritima*, and *Sonneratia apetala* based on the polarity of solvents and plant parts analyzed. These variations align with previously reported findings that solvent polarity, species-specific metabolic profiles, and plant organ type profoundly influence phytochemical yields (Chaves *et al*., 2020; Hira *et al*., 2024).

The phytochemical composition of true and mangrove associates showed significant variation both qualitative and quantitatively based on the polarity of the solvents and plant parts examined. Phytochemical yields are significantly affected by solvent polarity, species specific metabolic profiles, and plant part type (Kumar *et al*., 2023; Tourabi *et al*., 2023). In present study it is confirmed that methanol is a highly effective polar solvent for extracting phenolic compounds (Mohammed *et al*., 2022). Among the leaf samples, *S. apetala* recorded the highest phenolic content 41.21 mg/g, contrasting sharply with *D. trifoliata* hexane extract (3.67 mg/g). In case of stem (52.11 mg/g) and root samples (53.01 mg/g), *A. corniculatum* methanol extracts reported the highest phenolic levels, supporting earlier findings of Tripathi *et al*. (2025). Interestingly, *C. inerme* showed poor phenolic compounds recovery across all solvents, particularly in roots. This may be due to its limited biosynthetic capacity or reduced phenolics solubility in the solvents used (Zargoosh *et al*., 2019). Coumarins predominant in *A. corniculatum* ethyl acetate and methanol extracts. This is in agreement with findings of Sadeer *et al*. (2020). Conversely, *C. inerme* displayed minimal coumarin concentrations across all plant parts and solvents, potentially indicating tissue specific expression patterns of coumarin biosynthesis (Wang *et al*., 2025). The absence of coumarins in all aqueous stem extracts is consistent with their moderate polarity, which inherently limits their water solubility (Ihnatowicz *et al*., 2024). Glycosides were best extracted with methanol and ethyl acetate. Notably, *A. corniculatum* yields 6.89 mg/g of glycosides in its leaf methanol extract. This condition is aligns with its traditional medicinal importance (Kumar *et al*., 2013). In contrast, *D. trifoliata* accounted very few glycosides, especially in its root tissues, which could be due to either their localized presence. The poor extraction of glycosides in aqueous extract further confirms that water is not effectively isolating glycosides from the studied plant samples (Ren *et al*., 2020). In present study detectable levels of saponins were observed in all leaf samples studied. Methanol proved to be the most effective solvent for saponins extraction. The saponins content of *A. corniculatum* (21.41 mg/g) is significantly high in leaves and its roots showed an even higher 32.07 mg/g. This observation is in consistent with Mursaliyeva *et al*. (2023). Interestingly, no saponins were detected in *C. inerme* root extracts with non-polar solvents, which could indicate that saponins are localized to specific tissues in this plant or its saponin biosynthesis is limited (Paramitra *et al*., 2024). Our findings indicate that ethyl acetate and methanol are the optimal solvents for tannin extraction. We observed peak concentrations in *D. trifoliata* leaves (40.61 mg/g) and *A. corniculatum* stems (46.11 mg/g). The substantial presence of tannins in these plants cope with salt stress and defensive mechanisms (Campobenedetto *et al*., 2021). Conversely, the minimal extraction of tannins from aqueous and hexane extracts highlights their limited solubility (de Hoyos-Martínez *et al*., 2019). Optimum extraction of flavonoids was reported with ethyl acetate and methanol solvents. In present study highest flavonoid content 63.47 mg/g was observed in *A. corniculatum* leaf ethyl acetate extract. In contrast, hexane was unable to effectively extract flavonoids from the leaves of *D. trifoliata*, *S. apetala*, and *S. maritima*, indicating that the efficiency of flavonoid extraction is solvent polarity dependent (Tzanova *et al*., 2020). Notably, all root samples showed a consistent presence of flavonoids, indicating their stable synthesis and accumulation even in subterranean parts (Tripathi *et al*., 2025). Terpenoids were readily extracted with methanol and ethyl acetate, especially from *A. corniculatum* and *S. maritima*. The maximum content was found with *A. corniculatum* leaf methanol extract (48.12 mg/g) and stem (56.11 mg/g). Their widespread presence in root extracts supports their role in allelopathy and rhizospheric interactions (Gonzalez-Hernández *et al*., 2024). The aqueous extracts yielded less amounts of terpenoids, confirming aqueous medium less potent for terpene isolation. In present investigation alkaloids content was recorded to be high in the methanol root extracts of *S. maritima* (12.80 mg/g) and *A. corniculatum* (10.12 mg/g). This is further confirmed that their availability will enhance plant defensive mechanism (Lee *et al*., 2024). The absence of alkaloids in aqueous extracts of *C. inerme* and *D. trifoliata* roots indicates the significant role of solvent polarity on extraction efficiency of alkaloids. Furthermore, hexane extracts also showed poor alkaloid extraction, which are in accordance with Cravotto *et al*. (2022) in tropical halophytes.

4. Conclusion

Based on qualitative and quantitative screening, true mangrove ***Aegiceras corniculatum*** demonstrated a broad spectrum of phytochemical presence and recorded the higher yields for most bioactive compounds including phenolics (53.01 mg/g), coumarins (24.81 mg/g), flavonoids (63.47 mg/g), saponins (32.07 mg/g), tannins (51.17 mg/g), and terpenoids (56.11 mg/g) across methanol and ethyl acetate extracts. Among associate mangroves*,* ***Suaeda maritima*** outperformed the others. It showed more number of phytochemicals both qualitative and quantitatively. In *S. maritima* presence of phytochemicals such as saponins (18.23 mg/g), terpenoids (52.66 mg/g), flavonoids (16.32 mg/g), and alkaloids (6.87 mg/g) found to be high. These findings indicate that A. corniculatum and S. maritima possess superior phytochemical profiles, making them the most promising candidates among the examined true and associate mangroves for further pharmacological and nutraceutical studies.

References

Ajanal, M., Gundkalle, B. M., & Nayak, S. U. (2012). Estimation of total alkaloid in chitrakadivati by uv-spectrophotometer. Ancient Science of Life, 31(4), 198–201. <https://doi.org/10.4103/0257-7941.107361>. PMID: 23661869; PMCID: PMC3644759.

Alhakmani, F., Kumar, S., Khan, S. A. (2013). Estimation of total phenolic content, in-vitro antioxidant and anti-inflammatory activity of flowers of *Moringa oleifera*. Asian Pacific Journal of Tropical Biomedicine, 3(8):623-7; discussion 626-7. doi: 10.1016/S2221-1691(13)60126-4. PMID: 23905019; PMCID: PMC3703555.

Alongi, D. M. (2002). Present state and future of the world’s mangrove forests. Environmental Conservation, 29(3), 331–349. <https://doi.org/10.1017/S0376892902000231>.

Aryan, S., Mortazavian, A. M., Mohammadi, F., Mahdavi, V., Moazami, N., Jazaeri, S. (2022). Physicochemical properties of saponin containing *Acanthophyllum laxiusculum* extract: example application in foam stability and qualitative parameters for malt beverage industry. Journal of Food Science and Technology, 59(4):1577-1587. doi: 10.1007/s13197-021-05169-3. PMID: 35250081; PMCID: PMC8882519.

Atanasov, A. G., Zotchev, S. B., Dirsch, V. M., & Supuran, C. T. (2021). Natural products in drug discovery: Advances and opportunities. Nature Reviews Drug Discovery, 20(3), 200–216. https://doi.org/10.1038/s41573-020-00114-z.

Balakrishnan, R., Sangeetha, R., & Pandian, S. K. (2020). Mangroves as sources of bioactive natural products: A review. Biocatalysis and Agricultural Biotechnology, 23, 101472. https://doi.org/10.1016/j.bcab.2019.101472.

Bandaranayake, W. M. (2002). Bioactivities, bioactive compounds and chemical constituents of mangrove plants. Wetlands Ecology and Management, 10, 421–452. https://doi.org/10.1023/A:1021397624349.

Bibi Sadeer, N., Sinan, K. I., Cziaky, Z., Jeko, J., Zengin, G., Jeewon, R. et al. (2020). Assessment of the pharmacological properties and phytochemical profile of *Bruguiera gymnorhiza* (L.) Lam using in vitro studies, in silico docking, and multivariate analysis. Biomolecules, 10(5), 731. <https://doi.org/10.3390/biom10050731>.

Campobenedetto, C., Mannino, G., Beekwilder, J. et al. (2021). The application of a biostimulant based on tannins affects root architecture and improves tolerance to salinity in tomato plants. Scientific Reports, **11**, 354. <https://doi.org/10.1038/s41598-020-79770-5>.

Chaves, J.O., de Souza, M.C., da Silva, L.C., Lachos-Perez, D., Torres-Mayanga, P.C., Machado, APdF. et al. (2020). Extraction of flavonoids from natural sources using modern techniques. Frontiers in Chemistry. 8:507887. doi: 10.3389/fchem.2020.507887.

Cravotto C, Fabiano-Tixier AS, Claux O, Abert-Vian M, Tabasso S, Cravotto G. et al. (2022). Towards Substitution of Hexane as Extraction Solvent of Food Products and Ingredients with No Regrets. Foods, 11(21):3412. doi: 10.3390/foods11213412. PMID: 36360023; PMCID: PMC9655691.

de Hoyos-Martínez, P. L., Merle, J., Labidi, J., & Charrier – El Bouhtoury, F. (2019). Tannins extraction: A key point for their valorization and cleaner production. *Journal of Cleaner Production, 206*, 1138–1155. <https://doi.org/10.1016/j.jclepro.2018.09.243>.

Dey, P. M., & Harborne, J. B. (1989). Methods in Plant Biochemistry: Plant Phenolics. Academic Press.

Duke, N. C. (1992). Mangrove floristics and biogeography. In A. I. Robertson & D. M. Alongi (Eds.), Tropical Mangrove Ecosystems (pp. 63–100). American Geophysical Union.

GBD 2019 Collaborators. (2020). Global burden of 369 diseases and injuries in 204 countries and territories, 1990–2019: A systematic analysis. The Lancet, 396(10258), 1204–1222. https://doi.org/10.1016/S0140-6736(20)30925-9.

Gibbs R.D., 1974. Chemotaxonomy of Flowering Plants. Vol.1, McGill Queen’s University Press, Montreal and London.

Gonzalez-Hernández, R.A., Valdez-Cruz, N.A. & Trujillo-Roldán, M.A. Factors that influence the extraction methods of terpenes from natural sources. *Chem. Pap.* **78**, 2783–2810 (2024). <https://doi.org/10.1007/s11696-024-03339-z>.

Gurib-Fakim, A. (2006). Medicinal plants: Traditions of yesterday and drugs of tomorrow. Molecular Aspects of Medicine, 27(1), 1–93. https://doi.org/10.1016/j.mam.2005.07.008.

Harborne, J.B. (1973) Phytochemical Methods: A Guide to Modern Technique of Plant Analysis. Chapman and Hall, London, 107-150.

Hira, F. A., Islam, A., Mitra, K., Bithi, U. H., Ahmed, K. S., Islam, S., Abdullah, S. M. et al. (2024). Comparative analysis of phytochemicals and antioxidant characterization among different parts of *Catharanthus roseus*: In Vitro and In Silico investigation. Biochemistry Research International, 18; 2024:1904029. doi: 10.1155/2024/1904029. PMID: 39474335; PMCID: PMC11519068.

Igbinosa, E. O., Uzunuigbe, E. O., Igbinosa, I. H., Odjadjare, E. E., Igiehon, N. O., & Emuedo, O. A. (2013). In vitro assessment of antioxidant, phytochemical and nutritional properties of extracts from the leaves of *Ocimum gratissimum* (Linn). African Journal of Traditional, Complementary and Alternative Medicines, 10(5), 292–298. PMCID: PMC3847418, PMID: [24311838](https://pubmed.ncbi.nlm.nih.gov/24311838/).

IHME. (2019). India: Health Metrics. Institute for Health Metrics and Evaluation. Retrieved from http://www.healthdata.org/india.

Ihnatowicz, A., Siwinska, J., Perkowska, I. et al*.* (2024). Genes to specialized metabolites: accumulation of scopoletin, umbelliferone and their glycosides in natural populations of *Arabidopsis thaliana*. BMC Plant Biology, 24, 806. <https://doi.org/10.1186/s12870-024-05491-w>.

Kathiresan, K., & Bingham, B. L. (2001). Biology of mangroves and mangrove ecosystems. Advances in Marine Biology, 40, 81–251. https://doi.org/10.1016/S0065-2881(01)40003-4.

Kathiresan, K., & Rajendran, N. (2005). Coastal mangrove forests mitigated tsunami. Estuarine, Coastal and Shelf Science, 65(3), 601–606. https://doi.org/10.1016/j.ecss.2005.06.022

Kumar, A., P, N., Kumar, M., Jose, A., Tomer, V., Oz, E., Proestos, C., Zeng, M. et al. (2023). Major Phytochemicals: Recent advances in health benefits and extraction method. Molecules, 28(2), 887.

Kumar, K. S., Kumar, V. V., Prasanthi, A. G., Kanthal, L. K., Raju, P. B., & Satyavathi, K. (2013). Evaluation of cardiotonic activity of some traditionally used medicinal plants. *Research Journal of Pharmacology and Pharmacodynamics*, *5*(2), 87–91.

Lazarou, J., Pomeranz, B. H., & Corey, P. N. (1998). Incidence of adverse drug reactions in hospitalized patients. JAMA, 279(15), 1200–1205. <https://doi.org/10.1001/jama.279.15.1200>.

Lee, J.E., Jayakody, J. T. M., Kim, J.I., Jeong, J.W., Choi, K.M., Kim, T.S. et al. (2024). The Influence of Solvent Choice on the Extraction of Bioactive Compounds from Asteraceae: A Comparative Review. *Foods*, *13*(19), 3151. <https://doi.org/10.3390/foods13193151>.

Mohammed, E. A., Abdalla, I. G., Alfawaz, M. A., Mohammed, M. A., Al Maiman, S. A., Osman, M. A., et al. (2022). Effects of extraction solvents on the total phenolic content, total flavonoid content, and antioxidant activity in the aerial part of root vegetables. Agriculture, 12(11), 1820. https://doi.org/10.3390/agriculture12111820.

Muhammad, S. A., Abubakar, S .M. (2016). Qualitative and quantitative determination of phytochemicals in aqueous extract of *Chrysophyllum albidum* seed kernel. [Biosciences Biotechnology Research Asia,](file:///C:\Users\babuj\AppData\Roaming\Microsoft\Word\Biosciences%20Biotechnology%20Research%20Asia,) 13(2).<https://www.biotech-asia.org/?p=14064>.

Mursaliyeva, V. K., Sarsenbek, B. T., Dzhakibaeva, G. T., Mukhanov, T. M., & Mammadov, R. (2023). Total content of saponins, phenols and flavonoids and antioxidant and antimicrobial activity of *in vitro* culture of *Allochrusa gypsophiloides* (Regel) Schischk compared to wild plants. Plants, 12(20), 3521. doi: 10.3390/plants12203521. PMID: 37895985; PMCID: PMC10609880.

Paramita, O., Ansori, M., Wahyuningsih, Romiyatun, R. M. A., Ardelia, M. H., Sholeha, N. A., & Mayangsari, E. D. (2024). Identification of saponin compounds and antioxidant from Batu variety of longan leaf extract. *IOP Conference Series: Earth and Environmental Science, 1381*, 012042. <https://doi.org/10.1088/1755-1315/1381/1/012042>.

Polshettiwar, S. A., Ganjewale, R. O., Wadher, S. J., & Yeole, P. G. (2007). Spectrophotometric estimation of total tannins in some Ayurvedic eye drops. Indian Journal of Pharmaceutical Sciences, *69*(4), 574–576.

Rajat Buragohain. (2015). Screening and Quantification of Phytochemicals and Evaluation of Antioxidant Activity of Albizia chinensis (Vang): One of the Tree Foliages Commonly Utilized for Feeding to Cattle and Buffaloes in Mizoram. International Journal of Current Microbiology and Applied Sciences, 4 (9): 305-313.

Ren, Y., Tan, Q., Heath, K., Wu, S., Wilson, J. R., Ren, J., Shriwas, P., Yuan, C., Ngoc Ninh, T., Chai, H. B., Chen, X., Soejarto, D. D., Johnson, M. E., Cheng, X., Burdette, J. E., & Kinghorn, A. D. (2020). Cytotoxic and non-cytotoxic cardiac glycosides isolated from the combined flowers, leaves, and twigs of *Streblus asper*. *Bioorganic & Medicinal Chemistry*, *28*(4), 115301. doi: 10.1016/j.bmc.2019.115301. Epub 2020 Jan 7. PMID: 31953129; PMCID: PMC7029422.

Tomlinson, P. B. (2016). The Botany of Mangroves (2nd ed.). Cambridge University Press.

Tourabi, M., Metouekel, A., ghouizi, A.E.L. *et al.* (2023). Efficacy of various extracting solvents on phytochemical composition, and biological properties of *Mentha longifolia* L. leaf extracts. Scientific Reports, 13, 18028 (2023). <https://doi.org/10.1038/s41598-023-45030-5>.

Tripathi,. S, Singh, S., Mishra, N., Mishra, N. (2025). The impact of solvent polarity on the phenolic and antioxidant capacity of green coffee beans (*Robusta species*) extracts. Current Research in Nutrition and Food Science, 13(2). <https://bit.ly/3XJs66v>.

Truong, D. H., Ta, N. T. A., Pham, T. V., Huynh, T. D., Do, Q. T. G., Dinh, N. C. G. (2021). Effects of solvent-solvent fractionation on the total terpenoid content and in vitro anti-inflammatory activity of *Serevenia buxifolia* bark extract. Food Science and Nutrition. 9(3):1720-1735. doi: 10.1002/fsn3.2149. PMID: 33747483; PMCID: PMC7958534.

Tzanova, M., Atanasov, V., Yaneva, Z., Ivanova, D., & Dinev, T. (2020). Selectivity of Current Extraction Techniques for Flavonoids from Plant Materials. *Processes*, *8*(10), 1222. <https://doi.org/10.3390/pr8101222>.

Ventola, C. L. (2015). The antibiotic resistance crisis: Part 1: Causes and threats. Pharmacy and Therapeutics, 40(4), 277–283. PMCID: [PMC4378521](https://pmc.ncbi.nlm.nih.gov/articles/PMC4378521/).

Wang, Y., Guan, T., Yue, X., Yang, J., Zhao, X., Chang, A. et al. (2025). The biosynthetic pathway of coumarin and its genetic regulation in response to biotic and abiotic stresses. Frontiers in Plant Sciences,16:1599591. doi: 10.3389/fpls.2025.1599591.

World Health Organization. (2021). Traditional, complementary and integrative medicine. https://www.who.int/health-topics/traditional-complementary-and-integrative-medicine

Zargoosh, Z., Ghavam, M., Bacchetta, G. Ali Tavili. (2019).Effects of ecological factors on the antioxidant potential and total phenol content of *Scrophularia striata* Boiss. Scientific Reports,9, 16021. https://doi.org/10.1038/s41598-019-52605-8.